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PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Artcle 36 and Rule 70)

Applicant's or agent's file reference 2FPO-10-14	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. **PCT/KR2002/001975**	International filing date *(day/month/year)* **22 OCTOBER 2002 (22.10.2002)**	Priority date *(day/month/year)* 19 APRIL 2002 (19.04.2002)

International Patent Classification (IPC) or national classification and IPC

IPC7 G01N 33/68

Applicant

REGEN BIOTECH, INC. et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [X] Certain observations on the international application

Date of submission of the demamd	Date of completion of this report
23 SEPTEMBER 2003 (23.09.2003)	20 SEPTEMBER 2004 (20.09.2004)

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International aplication No.
PCT/KR2002/001975

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-49 , as originally filed
pages _____ , filed with the demand
pages _____, filed with the letter of _____

☒ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statment) under Article 19
pages _____ , filed with the demand
pages 50-54 , filed with the letter of 09/09/2004

☐ the drawings:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language English which is

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☒ the language of publication of the international application(under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide** and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained inthe international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disc losure in the international applicationas as filed has been furinshed.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box(Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed." and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION

International aplication No.
PCT/KR2002/001975

V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-16	YES
	Claims	none	NO
Inventive step (IS)	Claims	10-16	YES
	Claims	1-9	NO
Industrial applicability (IA)	Claims	1-16	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Reference is made to the following documents from the International Search Report (ISR).

D1: WO 96/01102

D2: EP 555989

D3: KR 1994-0026540 (KIST, Korea) 09 Dec 1994 : not listed in the Search Report.

Objects of the present invention are to provide a method (claims 1-9) to measure the amount of βig-h3 protein and a diagnostic kit (claims 10-16) using the same. The method comprises preparing recombinant βig-h3 proteins comprising 4th fas-1 domains as a standard protein, preparing specific ligand against the recombinant proteins and measuring the amount of βig-h3 protein of samples.

D1 is considered to represent the most relevant state of the art for the subject matter of present invention with respect to preparation and detection of a recombinant βig-h3 protein.

D2 relates to identification of a TGF-β induced gene encoding the βig-h3 protein but does not disclose a ligand to detect the βig-h3 protein.

(1) Novelty

(a) Regarding claims 1-9:

D1 discloses a recombinant βig-h3 protein, its specific ligand (antibody) and the detection method using antigen-antibody interaction. However, D1 differs from the subject matter of claim 1 in that it does not describe the use of the βig-h3 protein as a standard protein for quantitation. Therefore, claim 1 and its dependent claims 2-9 are considered novel meeting the criteria set forth in Article 33(2) PCT.

— Continued in Supplemental Box

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. Contrary to the requirements of Article 6 PCT, the following claims are not fully supported by the Description
(i) claims 1 & 5 : "specific ligand" and "antibody" are supposed to be prepared against recombinant β ig-h3 proteins comprising 4th fas-1 domains. However, example <1-3> describes that the primary antibody is raised against human β ig-h3 and mouse β ig-h3 proteins.

(ii) claims 2 & 11 : the disclosure is not sufficient for the subject matter of claims 2 & 11 regarding "the ligand", except for antibody. RNA, DNA and lipids are unlikely to function as a ligand of the β ig-h3 protein.

2. Contrary to the requirements of Article 6 PCT, "the ligand of step 1)" of claim 2 is not clear since the ligand is not found in step 1) of claim 1.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of:

Box V

(b) Regarding claims 10-16:
D1 discloses the use of the β ig-h3 protein to accelerate wound healing and inhibition of tumor cell growth in cells expressing the protein. D2 also describes relevance of the protein with some human cancers. In contrast, the subject matter of claim 10 relates to a kit comprising the β ig-h3 protein for diagnosing diseases such as renal diseases, hepatic diseases, rheumatoid arthritis and cardiovascular diseases but not cancers. It seems that there is no prior art relating β ig-h3 to the diseases described in claim 10. Accordingly, claim 10 and its dependent claims 11-16 are considered novel fulfilling the criteria set forth in Article 33(2) PCT.

(2) Inventive step

(a) Regarding claims 1-9:
The method of claim 1 appears to be a competitive assay using ligands(i.e., antibody) and standard proteins, which are identical to the β ig-h3 protein of samples or its parts. The β ig-h3 protein consists of several domains including the 4th fas-1 domain. The term "recombinant β ig-h3 proteins comprising 4th fas-1 domains" of claim 1 is interpreted as proteins encoded from the recombinant DNA construct carrying the full length or a part of the β ig-h3 gene.

The method disclosed in D1 is a simple immuno-detection method and different from that of the present invention, but D1 discloses all crucial materials for the method of claim 1 : a recombinant β ig-h3 protein, antibody and binding reaction. Furthermore, D3 discloses a method for measuring concentration of the apolipo-protein using a competitive enzyme-linked immunosorbent assay(ELISA) with purified apolipo-proteins as a standard. A skilled person in the art would consider measuring cellular levels of the β ig-h3 protein through a quantitative assay. Therefore, it is obvious that the state of the art would lead the skilled person to the combination of the features from D1 & D3.

Even in case the term "recombinant β ig-h3 proteins comprising 4th fas-1 domains" of claim 1 is interpreted as recombinant proteins of repeats of the 4th fas-1 domain, the use of the 4th fas-1 domain as a standard is not considered to involve an inventive step, because (i) the ligand was generated against the β ig-h3 protein not a single domain according to the Description, and (ii) there is no surprising effect of using the 4th fas-1 domain as a standard over using the β ig-h3 protein as described in the Description.

Claim 5 simply adds to claim 1 more features related to ELISA. Claims 2-4 & 6-9 are dependent on claim 1. The features of claims 2-9 are no more than what is disclosed in D1 & D3 or what is easily drawn from prior arts. Accordingly, claims 1-9 of the present invention do not fulfill the criteria set forth in Article 33(3)PCT for the lack of an inventive step.

(b) Regarding claims 10-16:
There is no lead in prior art for an ordinary skilled person in the art to consider the β ig-h3 protein as a diagnostic marker for renal diseases, hepatic diseases, rheumatoid arthritis and cardiovascular diseases. It is not obvious either. The subject matter of claim 10 is based on novel findings of the present invention and appears to involve an inventive step. Claims 11-16 are dependent on claim 1. Accordingly, claims 10-16 fulfill the criteria set forth in Article 33(3) PCT.

(3) Industrial applicability

The objectives of the present invention are to provide a protein measuring method and a diagnostic kit. There is no reason to negate the industrial applicability of this invention. Consequently, the claims 1~16 appear to meet the requirements of Article 33(4) PCT.

What is Claimed is

1. (amended) A method for measuring the amount of βig-h3 protein comprises the following steps:
   1) Preparing recombinant βig-h3 proteins comprising 4th fas-1 domains, their fragments or derivatives, as standard proteins;
   2) Preparing specific ligands against the above recombinant proteins, their fragments or derivatives of the above step 1; and
   3) Measuring the amount of βig-h3 protein of samples with the method using binding reaction of ligands of the above step 2 with the recombinant proteins, their fragments or derivatives of the above step 1.

2. The method for measuring the amount of βig-h3 protein as set forth in claim 1, wherein the ligands of step 1) are selected from a group consisting of antibodies, RNA, DNA, lipids, proteins, organic compounds and inorganic compounds.

3. The method for measuring the amount of βig-h3 protein as set forth in claim 1, wherein the

specific binding reaction of step 3) is antigen-antibody reaction.

4. The method for measuring the amount of βig-h3 protein as set forth in claim 3, wherein the antigen-antibody reaction is performed by a method selected from a group consisting of immunoblotting, immunoprecipitation, ELISA, RIA, protein chip, rapid assay and microarray.

5. (amended) The method for measuring the amount of βig-h3 protein as set forth in claim 3, wherein the antigen-antibody reaction of step 3) comprises the following steps:

1) Coating recombinant βig-h3 proteins comprising 4^{th} fas-1 domains, their fragments or derivatives to matrix;

2) Reacting antibody against the protein of the above step 1, its fragments or derivatives with sample;

3) Adding the reactant of the above step 2 to the coated protein of step 1 and waiting for reaction, and then washing thereof; and

4) Adding the secondary antibody to the reactant of the above step 3 for further reaction, and then measuring OD.

6. The method for measuring the amount of βig-h3 protein as set forth in anyone of claim 1-5, wherein the βig-h3 protein is human βig-h3 protein having amino acid sequence represented by SEQ. ID. NO 3 or mouse βig-h3 protein having amino acid sequence represented by SEQ. ID. No 5.

7. (amended) The method for measuring the amount of βig-h3 protein as set forth in anyone of claim 1-5, wherein the recombinant βig-h3 proteins comprising 4th fas-1 domains have 1-10 repeatedly-linked fas-1 domains.

8. The method for measuring the amount of βig-h3 protein as set forth in claim 7, wherein the fas-1 domain of βig-h3 is selected from a group consisting of sequences represented by SEQ. ID. No 7, No 8, No 9 and No 10.

9. The method for measuring the amount of βig-h3 protein as set forth in claim 1, wherein the sample can be any body fluid including urine, blood or synovial fluid.

10. A diagnostic kit for the renal diseases, hepatic

diseases, rheumatoid arthritis or cardiovascular diseases comprising βig-h3 protein or recombinant proteins of fas-1 domain in the βig-h3 protein (including their fragments or their derivatives) and their ligands.

11. The diagnostic kit as set forth in claim 10, wherein the ligand is selected from a group consisting of antibody specifically binding to β ig-h3 protein, fas-1 domain of βig-h3, their fragments or derivatives, RNA, DNA, lipids, proteins, organic compounds and inorganic compounds.

12. The diagnostic kit as set forth in claim 11, wherein the ligand is antibody.

13. The diagnostic kit as set forth in claim 12, wherein the kit additionally includes buffer solution, secondary antibody, washing solution, stop solution or coloring substrate.

14. The diagnostic kit as set forth in claim 10, wherein the βig-h3 protein is human βig-h3 protein having amino acid sequence represented by SEQ. ID. No 3 or mouse βig-h3 protein having

amino acid sequence represented by SEQ. ID. No 5.

15. The diagnostic kit as set forth in claim 10, wherein 1 or 2-10 4th fas-1 domains of βig-h3 protein are repeatedly linked.

16. The diagnostic kit as set forth in claim 15, wherein the fas-1 domain of βig-h3 is selected from a group consisting of sequences represented by SEQ. ID. No 7, No 8, No 9 and No 10.